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Immunoglobulin Responses in Acute Q Fever

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Knowledge of the development of different classes of antibody during the course of acute Q fever is important to the clinician for interpreting a patient's serological test results. In the present study, the appearance of antibodies to *Coxiella burnetii* phases I and II was determined for a period of 1 year. A total of 683 sera from 191 patients with symptomatic Q fever were evaluated by the complement fixation and indirect immunofluorescence (immunoglobulins M and G [IgM, IgG]) tests. These patients had contracted acute Q fever in the fall of 1983 during an epidemic that resulted in 415 serologically confirmed cases of Q fever. As demonstrated by the complement fixation test, antibodies to *C. burnetii* phase II remained elevated throughout the entire study period, whereas antibodies to phase I were barely detectable. Although the immunofluorescence test was more sensitive than the complement fixation test, the specific anti-IgG response to *C. burnetii* to phases I and II gave the same general antibody profiles as did the complement fixation test. IgM anti-phase I and II titers appeared earlier but disappeared after 10 to 12 weeks. During this period, anti-phase II antibody levels were generally much higher than anti-phase I antibody levels.

Acute Q fever is an influenza-like disease characterized by fever, pneumonia, headache, muscle cramps, arthritic pain, coughing, and general debility (1). In recent years, interest in Q fever has increased because of reports emphasizing the frequency and severity of the chronic forms of the disease, particularly endocarditis (8). Usually, acute Q fever is contracted by humans through inhalation of *Coxiella burnetii* in aerosols or in dust generated by domestic animals, particularly sheep. The disease is difficult to diagnose clinically, radiologically, or by traditional culture procedures. The diagnosis, however, can be made serologically, i.e., through the demonstration and titration of antibodies to the causative agent. Unique to *C. burnetii* is its antigenic phase variation (11). The virulent phase I is isolated from natural or laboratory infections of animals and humans, whereas the avirulent phase II occurs during serial passage of *C. burnetii* in immunologically incompetent hosts, such as embryonated chicken eggs or cell culture systems. Phase I to II variation seems to correlate with smooth and rough lipopolysaccharide changes (12). Specific high levels of anti-phase I antibodies are normally found in chronic Q fever (endocarditis) patients, whereas specific anti-phase II antibodies predominate during acute Q fever. As with many other infectious diseases, immunoglobulin M (IgM) antibodies are the first to appear. Usually they are detectable for a few weeks or, at the most, for a few months (4). IgG antibodies appear somewhat later but can persist for years, even for life (5).

Information on the nature (IgG and IgM) and development (anti-phase I, anti-phase II) of these antibodies is essential for the interpretation of serological results. The Q fever epidemic that occurred during the fall of 1983 in the Bagnes Valley of the state of Valais and resulted in 415 serologically confirmed Q fever cases (2) allowed us to study the development of the various antibodies for a period of 1 year. This outbreak started about 3 weeks after 12 flocks of sheep (between 850 and 900 animals) descended from the alpine pastures to the valley.

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MATERIALS AND METHODS

Serum samples from 191 of the 415 patients who had been seen by physicians and nurses in the Bagnes Valley or at the Martigny Hospital and for whom the time of onset of the symptoms was known were analyzed in the present study. Three or more blood samples were taken from each patient during a period of 1 year after onset. Thus, the 191 symptomatic patients included in our study provided a total of 683 sera. The remaining 224 patients who had not consulted a physician were not included in this study because they provided only two sera each. All 683 sera were evaluated for antibodies to *C. burnetii* phases I and II by complement fixation (CF) and indirect microimmunofluorescence (IF) tests (IgM, IgG). Negative and positive control sera were included in each test.

The titers of sera from the first 15 weeks after onset were averaged for each week and for each serological method. The titers obtained during weeks 16 to 20 and those obtained during weeks 21 through 26 were averaged for each of the two periods after onset. Last, 162 of the 191 patients provided a serum sample 52 weeks after onset.

Our file containing the characteristics of 191 patients was implanted in the Lausanne Polytechnical School computer (Control Data Cyber 855), and we mainly used SPSS (Statistical Package for the Social Sciences) and BMDP (Biomedical Computer Programs) for the study of the laboratory results.

CF test. The CF test micromethod was performed as described previously (Centers for Disease Control, Atlanta, Ga; 7), except that the starting serum dilution was 1:10 and the sensitized erythrocyte concentration was decreased to 2.5%. The *C. burnetii* phase I antigen was the Herzberg strain (Veterinaria AG, Zurich, Switzerland), and the *C. burnetii* phase II antigen was the Nine Mile strain (Virion AG, Ruschlikon, Switzerland). Serum, antigen, complement (Virion AG), and hemolytic system (Boehring, Marburg, Federal Republic of Germany) were prepared in Veronal buffer (BR16; Oxoid Ltd., London, England). The end point was defined as the highest dilution with ≥75% fixation.

IF test. The IF test was performed by the method of Philip

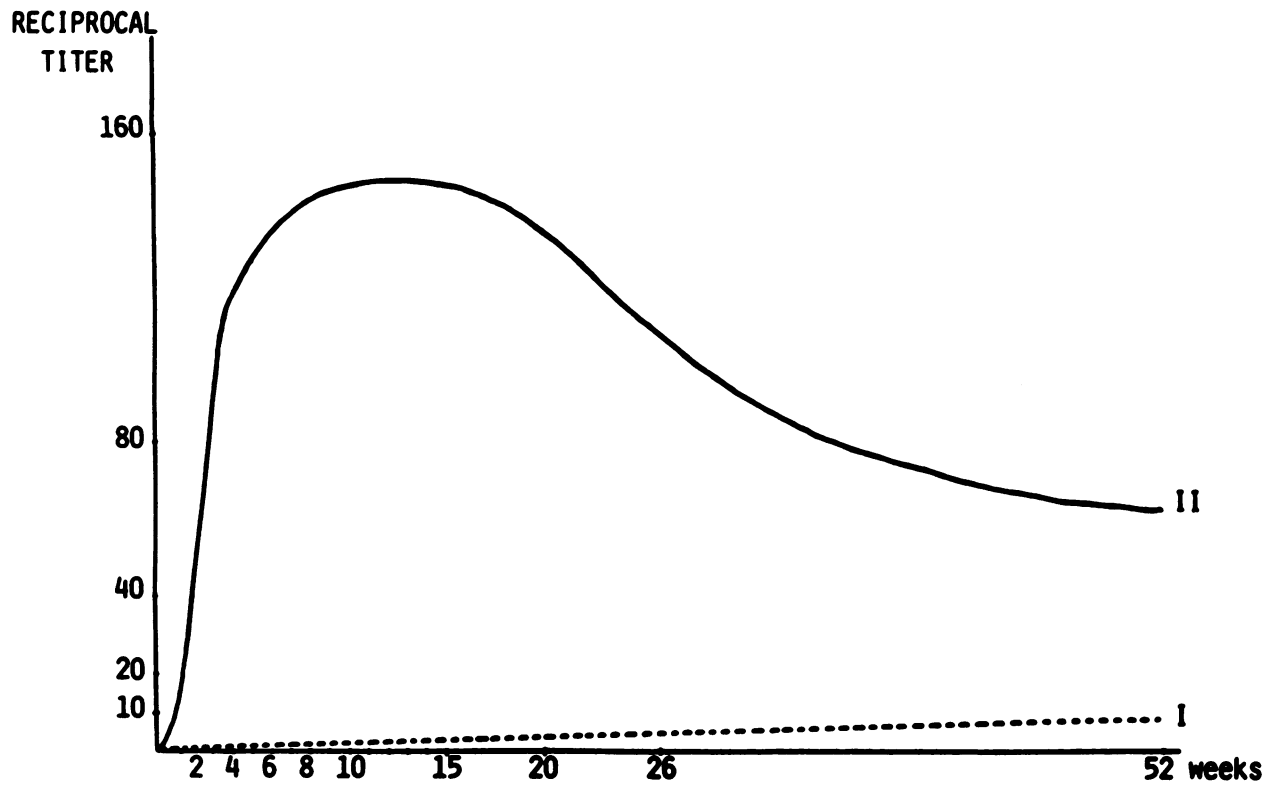

FIG. 1. Development of antibodies to *C. burnetii* phases I and II by CF test.

et al. (10). The *C. burnetii* phase I antigen (Nine Mile strain) had been passaged 307 times in guinea pigs, cloned from primary chicken embryo cell culture plaques, and passaged twice in chicken embryos. The *C. burnetii* phase II antigen (Nine Mile strain) had been passaged 90 times in chicken embryos, cloned from primary chicken embryo cell culture plaques, and passaged three times in chicken embryos. Antigens of *C. burnetii* phases I and II were applied to microscope slides with a pen over a premarked, 18-dot template. We used fluorescein isothiocyanate goat antihuman specific IgM and IgG conjugates (Biomérieux, Lyon, France). Sera from 50 patients showing discrepancies between CF and IF results were tested for rheumatoid factors with the Roche rheumatoid factor tube test (Roche Diagnostica, Basel, Switzerland).

RESULTS

In the first period (weeks 1 through 15) after onset, between 14 and 76 sera were received each week; in the second period (weeks 16 through 20), 22 sera were received; and in the third period (weeks 21 through 26), 14 sera were received. The last point on the graphs (see figures) represents the average antibody titer from the 162 sera obtained 52 weeks after onset. With these results we were able to draw a curve of average antibody titers to *C. burnetii* phases I and II for an entire year as measured by CF and IF (IgM, IgG).

In the CF test (Fig. 1), antibodies to *C. burnetii* phase II reached a reciprocal titer of 80 (lowest and highest: <10 to 640) by week 3 of illness. Peak titers averaging 160 (<10 to 1,280) were recorded between weeks 12 and 13. At 6 months, the average antibody titer was still 100 (<10 to 640), and after 1 year it was 60 (<10 to 640). Of 162 sera tested 1 year after the onset of illness, 13 (8%) had no detectable antibodies, whereas 33 (20%) still had a titer of 80 or greater. During the acute stage of illness, anti-phase I antibodies were not detected by CF; 1 year later, the average titer was 8 (<10 to 20).

The IF test detected IgM antibodies already at a titer of 40 (<20 to 320) against *C. burnetii* phase II in week 1 (Fig. 2). Titers reached a peak of 700 (<20 to 5,120) by week 3 but then dropped to a level of 20 (<20 to 80) by week 14. Anti-phase I IgM behaved in practically the same way, although titers were lower, 200 (<20 to 640), by week 3. None of the 50 sera tested for rheumatoid factors were positive.

Anti-IgG titers to *C. burnetii* phase II averaged 840 (80 to 2,560) by week 3 (Fig. 3) and reached a maximum titer of 1,000 (320 to 5,120) by week 8. At 1 year after the onset of illness, the average titer was still 700 (<20 to 5,120), although 1.2% of the sera had no detectable antibodies. Against *C. burnetii* phase I, the titers averaged 20 (<20 to 160) by week 5 and 50 (<20 to 640) about 1 year later.

DISCUSSION

The large number of patients surveyed for 1 year after their attack of acute Q fever allowed us to assess the serological profiles of the different antibodies during the course of the disease. The correct interpretation of the results, however, demands that the advantages and limitations of the techniques used be taken into consideration.

Our results with the most routinely used CF test, in which *C. burnetii* phase II is the antigen, show that antibodies reached maximum titers by week 12 and then declined throughout the year. At that time, however, 20% of our patients had titers equal to or greater than 80. These findings clearly suggest that it is not possible to retrospectively diagnose a Q-fever-like illness based on a single titer. The

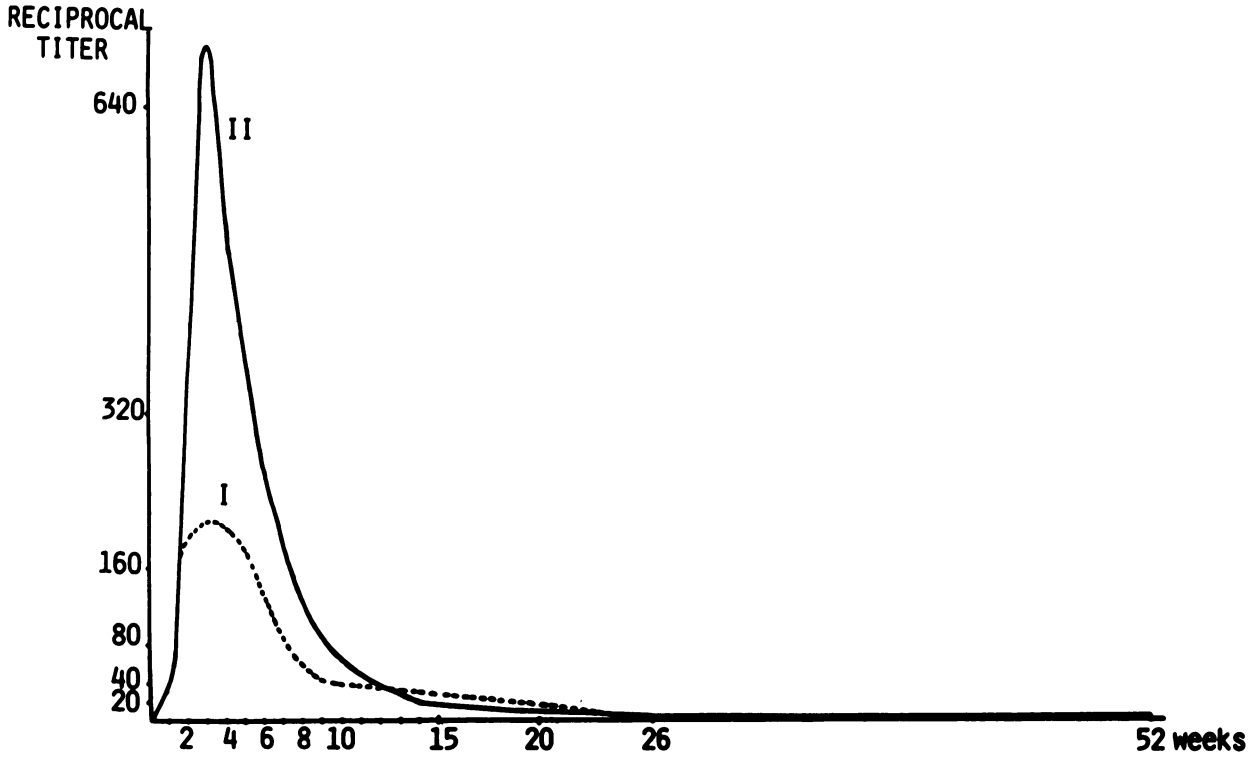

FIG. 2. Development of IgM antibodies to *C. burnetii* phases I and II by IF.

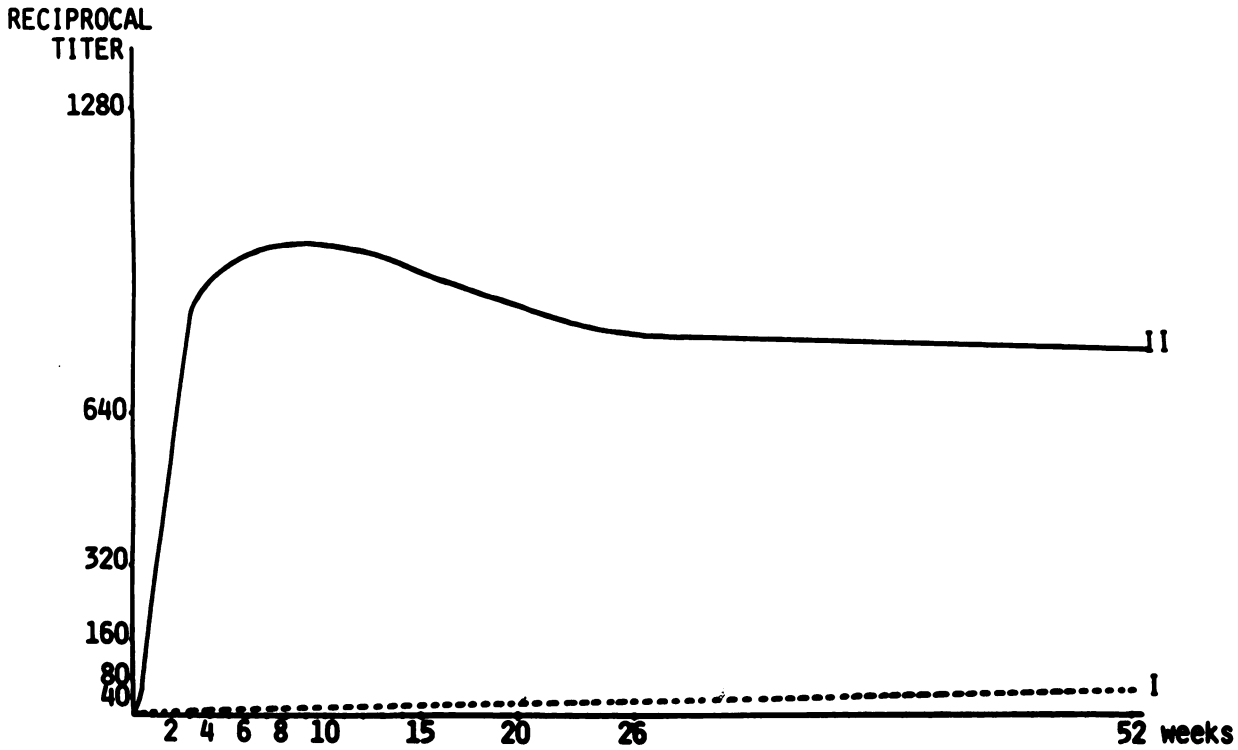

FIG. 3. Development of IgG antibodies to *C. burnetii* phases I and II by IF.

best criterion for a dependable diagnosis is still the demonstration of a fourfold or higher increase in antibody titer between the acute and convalescent serum samples.

CF antibodies to *C. burnetii* phase I are essentially negative during the year after the onset of acute Q fever. A titer equal to or greater than 200, on the other hand, may be diagnostic for chronic Q fever endocarditis (8).

IF is an easy and rapid test for the identification of specific IgM antibodies that appear to persist for as long as 11 to 17 weeks after onset (3, 6). In our study, they were present during week 1, reached maximum titers by week 3, and persisted for about 12 weeks. Although retrospective analysis of a single serum can be diagnostic, it should be noted that in our study, 5 of 162 patients (3%), even after 52 weeks, had significant IgM titers, which may complicate an accurate diagnosis. The clinical significance of these residual IgM antibodies is not known.

The serological profile of IF-tested anti-phase I IgM is similar to that of phase II, but with much lower titers. In convalescent sera, the phase I titers were usually not significant.

Although the IF test was more sensitive than the CF test, the specific anti-IgG response to phase I and II *C. burnetii* gave the same general antibody profiles as did the CF test. However, the IF phase II titers, after reaching a maximum by week 8, declined somewhat more slowly than the CF titers. Therefore, for a retrospective diagnosis, the same restrictions are relevant as for the CF test.

With the IF test, it should be remembered that in Q fever endocarditis, both phase I and phase II antibody levels (IgG, IgM) are elevated, and anti-phase I titers tend to be greater than the anti-phase II titers (9).

Because of the stability and the cooperation of the patients involved in our study, we hope to be able to do a comprehensive serological follow-up annually. In this way, we might be in a position to determine the presence of chronic Q fever in the early clinical stages of disease. However, we are well aware that some patients may continue to be exposed to *C. burnetii*, which may alter the antibody profile.

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